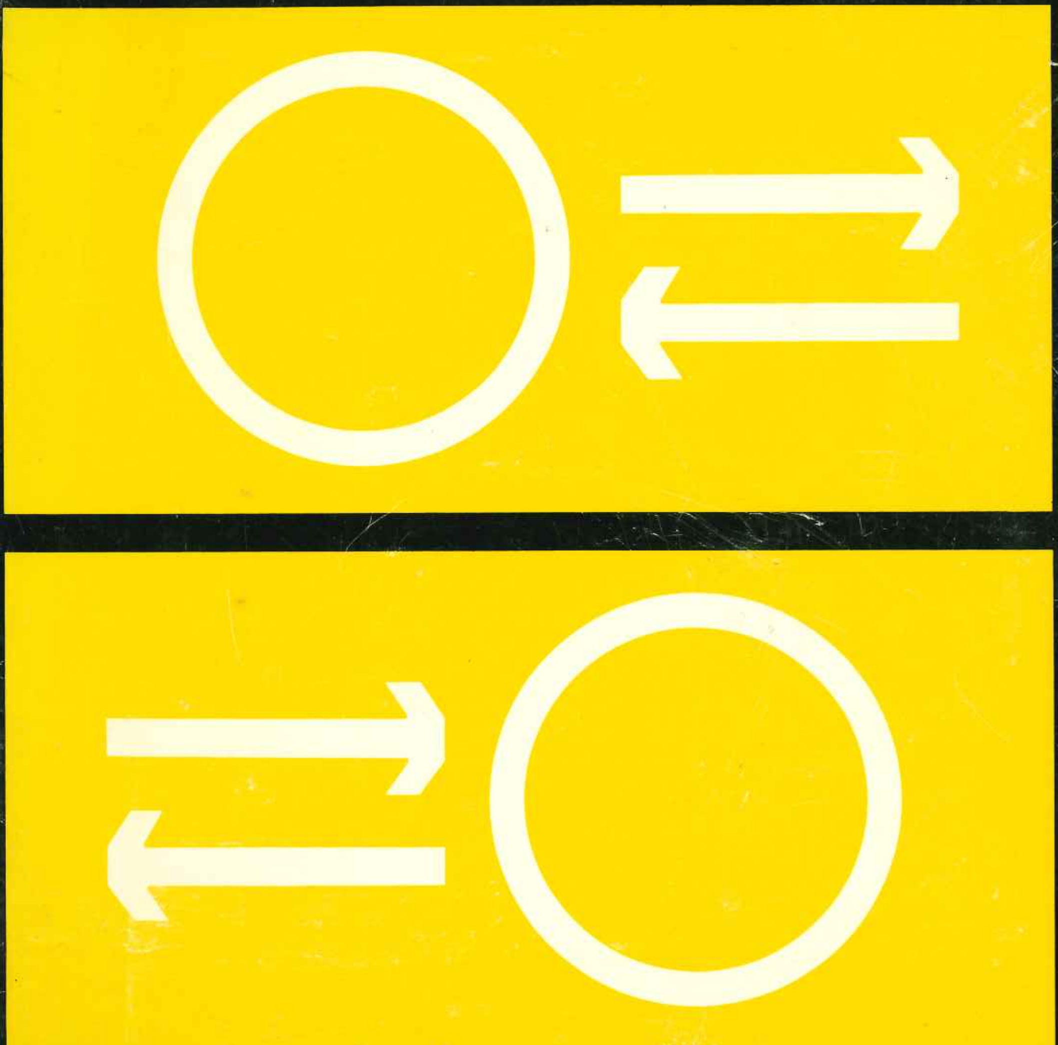
DEAE-Sephacel®

Beaded cellulose ion exchanger



DEAE-Sephacel®

- DEAE-Sephacel® is the only cellulose-based ion exchanger with the hydrodynamically correct spherical form.
- No precycling is required; DEAE-Sephacel is supplied fully swollen.
- DEAE-Sephacel is easy to pack to give chromatographic beds with excellent flow properties and without the risk of clogging by fines.
- The bead form and rigidity of DEAE-Sephacel prevent the generation of fines usually associated with conventional cellulose ion exchangers.
- Bed volume changes during elution with either pH or ionic strength gradients are minimal.
- The pore structure is carefully controlled and is practically unaffected by changes in the ionic strength or pH of the eluent.
- DEAE-Sephacel has a high capacity for proteins and other large molecules.
- DEAE-Sephacel is reproducible from batch-to-batch.
- Non-specific adsorption is low and recoveries are close to 100 %.

Introduction

The introduction of cellulose-based ion exchangers in 1956 was an important breakthrough in the development of chromatographic materials which could be used with proteins and other labile biological substances. The lack of non-specific adsorption and the porous structure of the cellulose derivatives developed by *Peterson* and *Sober* (1) contrasted strongly with the properties of resin-based ion exchangers previously available. The early commercially available cellulosic ion exchangers were, unfortunately, extremely variable. Their fibrous form made them difficult to pack and flow rates were often very low. More seriously, their degree of substitution was variable and different batches of apparently the same material gave widely differing results. The chemical reproducibility of cellulose-based ion exchangers has improved in recent years, but not until the preparation of bead-formed DEAE-, CM-, and SP-celluloses by *Determann, Meyer* and *Wieland* (2) was there any great progress in improving their physical form.

The importance of the physical form of cellulose ion exchangers has been discussed by *James* and *Stanworth* (3). The coarse fibrous form typical of many ion exchange celluloses was considered to increase disturbances caused by channelling and longitudinal diffusion thus reducing the efficiency of the chromatographic bed (3). The bead form is essential to chromatographic bed materials if the best possible hydrodynamic properties are required, and at the same time gives the important practical advantages of ease of packing and freedom from fines generation. DEAE-Sephacel is the only commercially available cellulose ion exchanger which is bead formed. It is ideal for consistent, high resolution chromatography of proteins, nucleic acids, hormones and other biopolymers.

Structure and functional groups

Cellulose is a naturally occurring polysaccharide consisting of β (1 → 4) linked glucose units. The usual sources are wood or cotton fibres. In the native state, adjacent polysaccharide chains are extensively hydrogen-bonded forming microcrystalline regions. These regions are interspersed by amorphous regions with less hydrogen-bonding. Limited acid hydrolysis results in preferential loss of the amorphous regions and gives so-called microcrystalline cellulose.

DEAE-Sephacel is produced from high purity, microcrystalline cellulose from wood fibres. During production the microcrystalline structure is broken down, and the cellulose is regenerated to give a bead-formed gel-like structure. The wet bead diameter is

40–160 μm. This gel is strengthened by cross-linking with epichlorohydrin, although the main structure-forming bonds are still hydrogen bonds. The functional groups are attached by ether linkages directly to the glucose units of the polysaccharide chains. The whole process is carefully controlled to give a high degree of reproducibility both with regard to structure and degree of substitution.

The diethylaminoethyl groups have the structure $-\ C_2H_4\overset{+}{N}H\ (C_2H_5)_2$. The gel is supplied with chloride counterions.

DEAE-Sephacel is prepared with the care and attention necessary to ensure good batch-to-batch reproducibility. Each batch is tested for function and capacity. It is supplied pre-swollen in suspension in NaCl solution (0.5 M) containing 25 % denatured ethanol to prevent microbial growth.

Properties

Swelling

DEAE-Sephacel is supplied pre-swollen and the degree of swelling is almost independent of the ionic strength or pH of the medium. Changes in bed volume during a chromatographic run are very small, ca 5 % between $I = 0.05$ and $I = 0.5$.

Capacity

The capacity of any ion exchanger is determined partly by the characteristics of the ion exchanger, e.g. its charge density and porosity, and partly by the properties of the exchanging ion, e.g. its charge characteristics and size. The total capacity of DEAE-Sephacel is 1.4 ± 0.1 meq/g dry wt and its capacity for serum albumin is about 150 mg/ml. The exclusion limit for dextran is approximately 300,000 daltons which corresponds to an exclusion limit for globular proteins of about 1×10^6 daltons. The adsorption kinetics of cellulose gel ion exchangers are substantially the same as for conventional cellulose ion exchangers (2).

Capacity in an ion exchange system is also a function of the ionic composition of the eluent. The variation of capacity with pH depends on the ionization constants of both the ion exchanger and the solutes. The titration curve for DEAE-Sephacel is shown in Figure 1. Side reactions which occur during the introduction of the DEAE-groups are responsible for the small number of charged groups, $pK_a \sim 7$.

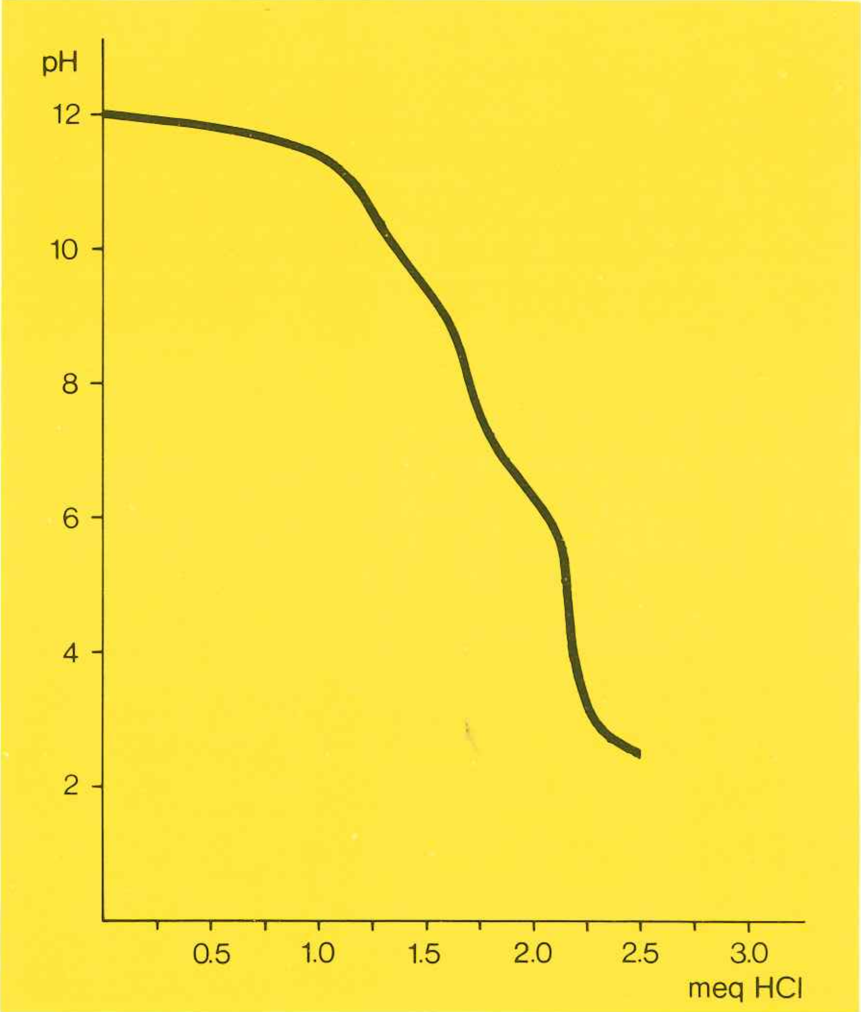


Figure 1. Titration of 1 g DEAE-Sephacel in 1 M KCl.

Stability

DEAE-Sephacel is stable in aqueous suspension within the pH range 2–12. Hydrolysis may occur in strongly acidic solutions and the macromolecular structure is broken down in strongly alkaline solutions. Please note that the free base form of DEAE-ion exchangers which is produced at high pH is unstable. Strong oxidizing agents should also be avoided.

DEAE-Sephacel, like all cellulose ion exchangers, is liable to microbial degradation, particularly in the presence of phosphates. Hibitane® (0.002 % as chlorhexidine) and Chloretone® (0.05 %) are effective antimicrobial agents for anion exchangers. Enzymes which hydrolyse β-glucosidic linkages may also cause biological degradation of DEAE-Sephacel, and it may be preferable to chromatograph samples suspected of containing such enzymes on Sephadex® ion exchangers or Sepharose® ion exchangers.

DEAE-Sephacel may be autoclaved at pH 7 for 30 minutes at 120 °C. During autoclaving minute quantities of carbohydrate are released: these can be washed away with sterile buffer solution.

Experimental technique

The spherical form and high rigidity of DEAE-Sephacel make it very easy to work with and give it excellent chromatographic properties. The experimental methods suitable for use with DEAE-Sephacel are broadly similar to those used with ion exchangers based on Sephadex or Sepharose. Please consult the booklet "Sephadex ion exchangers. A guide to ion exchange chromatography" for a more thorough discussion of the general methodology of ion exchange chromatography. Factors which should be especially born in mind when working with DEAE-Sephacel are described below.

Choice of ion exchanger

DEAE-Sephacel is suitable for ion exchange chromatography of solutes which are stable at pH values above their isoelectric points i.e. when the solutes are negatively charged. Solutes which are only stable in their cationic form should be chromatographed on cation exchangers such as CM-Sephadex, SP-Sephadex or CM-Sepharose. As can be seen from its titration curve (fig. 1), DEAE-Sephacel has a good capacity over the range pH 2–9. For anion exchange chromatography at pH values up to 10, a strong anion exchanger, e.g. QAE-Sephadex, should be used.

Preparation of DEAE-Sephacel

Equilibration

DEAE-Sephacel is supplied fully swollen and does not have to be precycled with acid and alkali as do conventional dry cellulose ion exchangers. It is, however, essential that the ion exchanger be in complete ionic equilibrium with the starting buffer before the sample is applied. The procedure for equilibration depends on whether or not the chloride counterion is to be retained. If the starting buffer contains only chloride counterions, e.g. Tris/HCl buffer solutions, the required amount of DEAE-Sephacel is simply stirred into an excess of starting buffer. The supernatant should be decanted and replaced with fresh buffer several times until its pH remains constant.

If the counterions are to be changed, e.g. when using acetate buffer solutions, the ion exchanger should be suspended in an excess of 0.5–1.0 M solution of a salt of the new counterion. After the gel has sedimented and the supernatant has been decanted, the ion exchanger must be thoroughly equilibrated with the starting buffer solution to be used. Equilibration with buffer solutions which contain buffering counterions, e.g. acetate and phosphate buffers, should be carried out with the greatest care if reproducible results are to be obtained and, if possible, such buffers should be avoided.

Drying and reswelling

Although it is not recommended that DEAE-Sephacel be dried, this may be required in special circumstances. After washing with distilled water, DEAE-Sephacel may be dried by freeze drying or by dehydration with successive additions of ethanol solutions of increasing concentration up to 95 %. When DEAE-Sephacel is dried, hydrogen bonding between the hydroxyl groups of adjacent polysaccharide chains is increased so much that resuspension in buffer solution is insufficient to reswell the cellulose matrix completely. If dried gel is to be reused for chromatography, it must be treated successively with 0.1 M HCl and 0.1 M NaOH.

Stir the ion exchanger into excess HCl (0.1 M, 20 ml/g dry DEAE-Sephacel) and allow it to swell for 30 minutes. Filter off the supernatant, wash with distilled water and add NaOH solution (0.1 M, 20 ml/g dry DEAE-Sephacel). After 30 minutes, filter off the supernatant and repeat the alkali treatment. Finally wash well and equilibrate with the starting buffer. Note that DEAE-Sephacel is in the free base form after the alkali treatment. It should be converted to the appropriate salt form as soon as possible to avoid loss of DEAE-groups.

Column chromatography

Choice of column

At the start of an ion exchange chromatography experiment the substances are usually absorbed within the upper 2 cm of the bed, and small columns are often sufficient. Pharmacia columns K 16 and K 26 should be used for bed volumes up to 500 ml and bed heights up to 100 cm. The use of flow adaptors A 16 or A 26 greatly simplifies sample application and allows the use of upward flow elution.

Preparing the chromatographic bed

Mix the equilibrated ion exchanger with starting buffer to give a slurry which is just thin enough to allow air bubbles to rise through it easily. 1 g gel slurry as packed gives a bed volume of 0.8–0.85 ml. Pack the column in the usual way using a Gel and Eluent Reservoir to extend the column for one-step packing. Pack the column at a somewhat higher operating pressure than that to be used in the chromatographic stage.

After packing, the bed should be stabilized by allowing one or two bed volumes of starting buffer to run through at the operating pressure to be used in the subsequent chromatographic step. Check that the pH and conductivity of the effluent are the same as those of the starting buffer, indicating that the bed has reached proper equilibrium.

Sample

The amount of sample applied should not normally exceed 10 % of the available bed capacity (see page 3). If all substances of interest are adsorbed under the starting conditions, the sample volume is of little importance in comparison to the amount of solute present. The ionic composition of the sample should be adjusted to be the same as that of the starting buffer. This is usually done most conveniently and rapidly by gel filtration on Sephadex G-25, G-15 or G-10. Prepacked columns PD-10 will be found particularly convenient for buffer exchange of sample volumes up to 2.5 ml using Sephadex G-25.

Elution

Bound substances are eluted by changing the pH or ionic strength of the eluent. Gradient elution is preferable to stepwise elution at the start of a series of experiments, since the artifacts often associated with stepwise elution of poorly characterized mixtures can then be avoided. The Pharmacia Gradient Mixer GM-1 will provide reproducible, linear ionic strength gradients with a total volume of up to 500 ml. The Pharmacia Peristaltic Pump P-3 provides linear, concave and convex ionic strength gradients of unlimited volume. Bed volume changes during gradient elution are minimal with DEAE-Sephacel.

Since the total capacity of DEAE-Sephacel is less than that of DEAE-Sephadex, substances will be eluted from DEAE-Sephacel at lower ionic strengths. Substances which are readily denatured in low ionic strength solutions should be chromatographed on Sephadex ion exchangers.

Flow rate

The rigidity of DEAE-Sephacel enables high flow rates to be used, and the maximum flow rate of which a bed is capable is not normally reached in practice. Figure 2 shows the flow rates obtained in standard laboratory columns. Linear flow rates up to 10 cm/hour are usually suitable for the resolution of protein mixtures using continuous gradients. Higher flow rates may be used with step gradients. For applications which require exceptionally high flow rates use DEAE-Sepharose CL-6B.

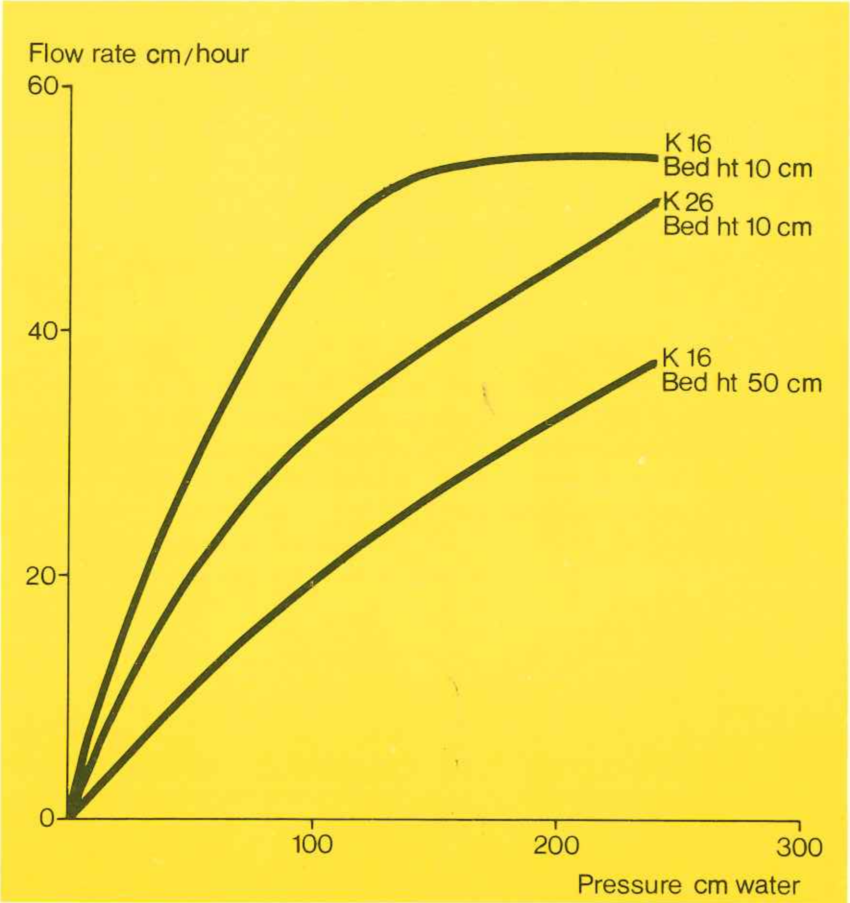


Figure 2. Flow rates as a function of the pressure drop across beds of DEAE-Sephacel. Tris-HCl buffer solution (0.1 M, pH 7.6).

Storage

DEAE-Sephacel should be stored as a thick slurry in its salt form. Ethanol (25 %), Hibitane (0.002 % as chlorhexidine) or Chloretone (0.05 %) should be added to prevent microbial growth.

Applications

Applications of ion exchange chromatography can be found in all fields of biochemistry. The separations described below are typical of the results obtained in our laboratories with DEAE-Sephacel in fractionations of serum proteins, enzymes and mononucleotides.

Serum proteins

Mammalian serum proteins may be fractionated on DEAE-Sephacel using either an ionic strength gradient, a pH gradient or a combination of both. The fractionation of human serum proteins using DEAE-Sephacel and a combined pH and ionic strength gradient is shown in Figure 3. Fresh human serum 2.5 ml was transferred to the starting buffer by buffer exchange on Sephadex G-25 (Pharmacia prepacked column PD-10). The protein fraction (3 ml) was applied to a column (K16/20) packed with DEAE-Sephacel and eluted with a concave gradient (total volume 360 ml) from Tris-phosphate (pH 8.4, 0.04 M) to Tris-phosphate (pH 3.4, 0.5 M). The fractions indicated were analysed by electrophoresis in Polyacrylamide Gradient Gels PAA 4/30 (fig. 4).

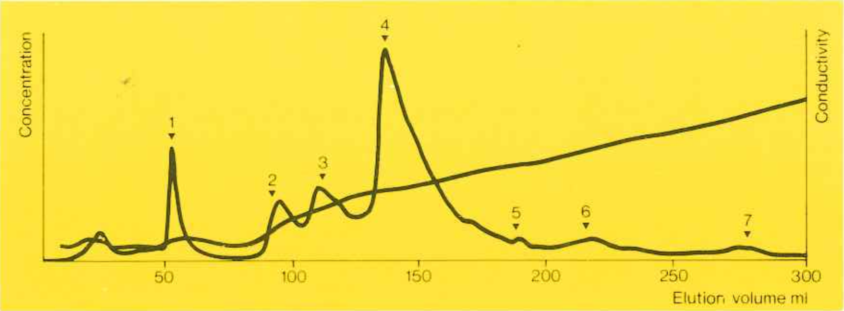


Figure 3. Fractionation of serum proteins on DEAE-Sephacel. Sample, human serum; column K 16/20, bed height 10 cm; eluent, Tris-phosphate (0.04 M, pH 8.4) with concave gradient to Tris-phosphate (0.5 M, pH 3.4); flow rate, 0.25 ml/min (7.5 cm/h). The compositions of the peaks indicated were examined by gradient gel electrophoresis (fig. 4).

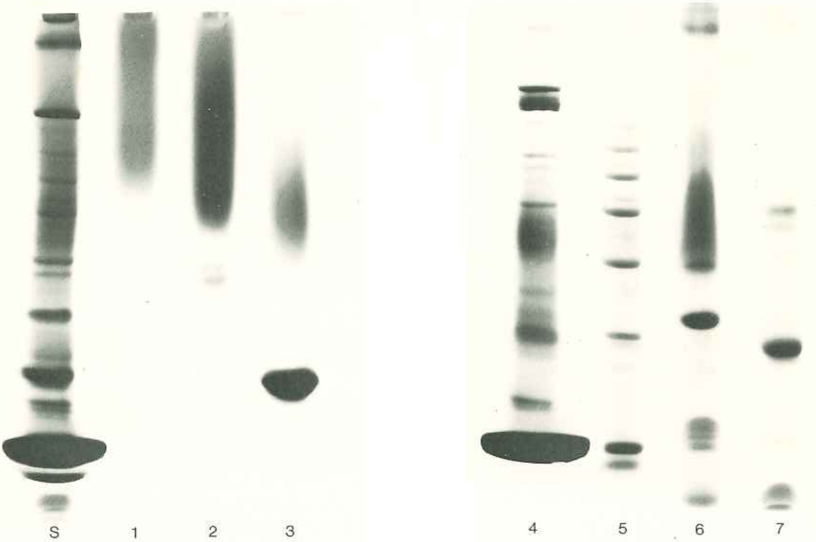


Figure 4. Electrophoresis of human serum and fractions from the separation shown in Figure 3. Gel: Pharmacia Gradient Gel PAA 4/30. The numbers correspond to the peaks in Figure 3. Track S is serum; 1 and 2, IgG; 3, transferrin; 4, albumin; 5, haptoglobins; 6, anti-thrombin III, α_1-antitrypsin, β_1-globulin, C-3 component; 7, ceruloplasmin.

Bacterial cytochromes

Cytochromes from *Pseudomonas aeruginosa* may be fractionated by a combination of anion exchange and cation exchange chromatography. A crude extract of *Ps. aeruginosa* was first concentrated on CM-Sepharose CL-6B by adsorption in ammonium acetate buffer (0.05 M, pH 3.9) and stepwise elution with ammonium acetate buffer (0.10 M, pH 6.5). Fractions containing the cytochromes were pooled. A sample was transferred to Tris-HCl buffer (0.01 M, pH 8.5) by buffer exchange on Sephadex G-25 (Pharmacia prepacked Column PD-10) and applied to a column packed with DEAE-Sephacel equilibrated with the same buffer solution. The cytochromes were separated by elution with a linear gradient (total volume 200 ml) from Tris-HCl buffer (0.01 M, pH 8.5) to Tris-HCl buffer (0.01 M, pH 8.5) containing NaCl (0.125 M). The results are shown in Figure 5.

Adenosine phosphates

DEAE-Sephacel gives excellent results in the separation of small molecules as well as proteins and other biopolymers. The resolution of a mixture of AMP, ADP and ATP using a simple linear ionic strength gradient is a typical example (fig. 6).

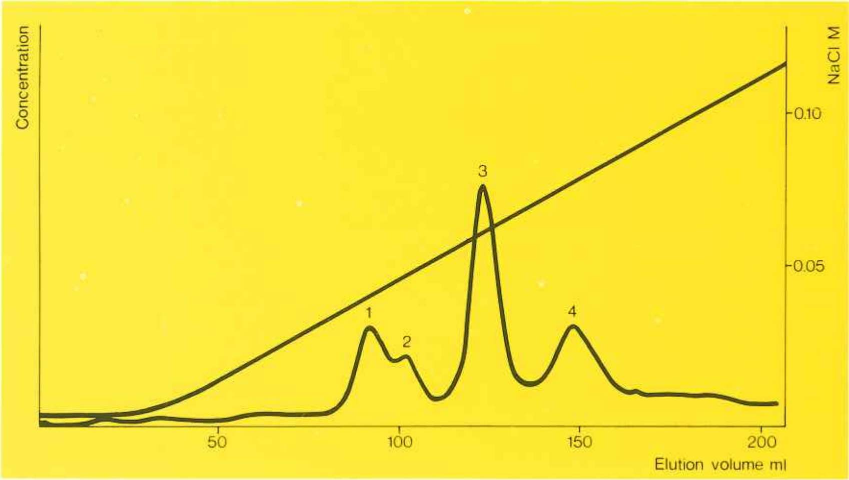


Figure 5. Fractionation of bacterial cytochromes on DEAE-Sephacel. Sample, see text; Column K 16/20, bed height 14.5 cm; eluent, Tris-HCl (0.01 M, pH 8.5) with linear concentration gradient of NaCl (0–0.125 M); flow rate 0.35 ml/min (10.5 cm/h). Peak 2 was azurin and the main peak (3) was tentatively assigned to cytochrome c 551. Peaks 1 and 4 were haem-containing proteins but were not identified.

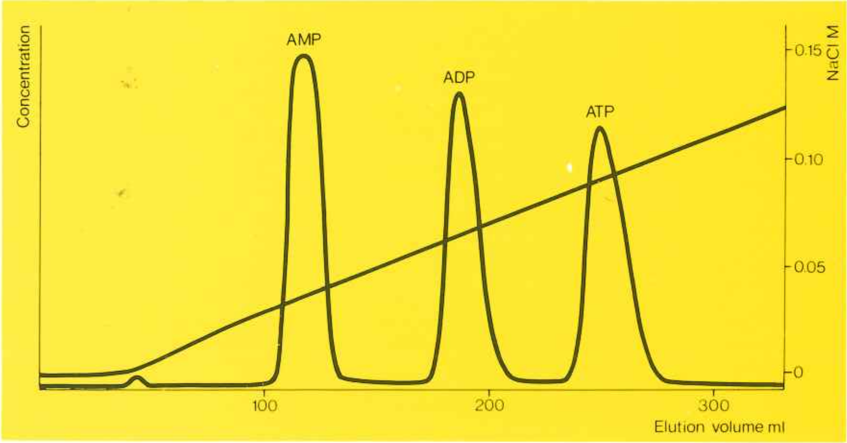


Figure 6. Separation of AMP, ADP and ATP on DEAE-Sephacel. The sample (3 ml) contained 4 mg of each nucleotide; column K 16/20, bed height 17.5 cm; eluent, Tris-HCl (0.1 M, pH 7.6) with a linear gradient of NaCl concentration (0–0.15 M); flow rate, 0.83 ml/min (25 cm/h).

Availability

DEAE-Sephacel is available in packs containing approximately 1 litre sedimented gel as a suspension in 25 % ethanol.

References

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